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Proton-Fueled, Reversible Assembly of Gold Nanoparticles by Controlled Triplex Formation**

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Since the first reports on gold nanoparticles (AuNPs) bearing oligonucleotides (DNA–AuNP conjugates) in 1996,[1] these conjugates have been used intensively to generate self-assembled, networked structures of AuNPs as well as to monitor hybridization/denaturation of oligonucleotides.[1,2] Previously reported approaches to the network formation of AuNPs were generally based on the hybridization of complementary oligonucleotides conjugated to AuNPs, and the one-directional assembly of AuNPs was mainly used for nanometer-scale structuring and biosensing (the disassembly occurred by heating).[2] However, more applications would be envisioned when the programmable, reversible assembly of AuNPs is achieved. To demonstrate reversible assembly/disassembly of DNA–AuNP conjugates, Niemeyer and co-workers used a fueling oligonucleotide that contained a short, dangling-end sequence for the initiation of strand removal from the assembled AuNP structures.[3a] A simpler approach would be the use of protons (H^+) as a fuel instead of oligonucleotides to control the structure of DNA and consequently the assembly/disassembly of DNA–AuNP conjugates. The advantages of protons over oligonucleotides have been suggested, including no generation of waste duplex products that poison DNA-based systems.[3b]

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Triple-helix DNA (triplex) is a well-documented structure in biology that is formed by the association of a target duplex and a homopyrimidinic "triplex-forming oligonucleotide".[4] Depending on the orientation of the third oligonucleotide with respect to the targeted Watson–Crick duplex, triplexes are classified into parallel and antiparallel structures. The formation of antiparallel triplexes does not require protonation and exhibits pH-independent binding. In contrast, parallel triplexes are critically dependent on the protonation of the imino group (N3) of cytosines at the triplex-forming oligonucleotide. The imino group should be protonated to form the correct Hoogsteen binding with N7 of guanine, and therefore the formation of parallel triplexes can be tuned dynamically by controlled changes in pH value. In other words, parallel triplexes are stable only under acidic conditions and the formation of an oligonucleotide-based triplex provides a powerful tool for genetic manipulation and other applications, such as DNA-based nanomachines.[5–7] We reasoned that the required protonation of the imino group (N3) of cytosines could be utilized for proton-fueled, reversible assembly/disassembly of AuNPs conjugated with oligonucleotides that form triplex structures ("triplex–AuNP conjugates").[8] Herein, we report that the proton-fueled switching of the assembly of AuNPs is realized by the pH-controlled fine-tuning of intermolecular interactions between two triplex-forming oligonucleotide parts.

We formed the triplex–AuNP conjugates by using two different thiolated, single-stranded oligonucleotides, oligo A and oligo B. Oligo A contained a sequence that formed a Watson–Crick hairpin duplex, and oligo B contained a triplex-forming oligonucleotide sequence (Figure 1).[8] Oligo A was a 5′-thiolated oligonucleotide composed of four sections: a 20-nucleotide poly-dA section starting from the 5′ terminus to increase hybridization efficiency,[9] a 12-nucleotide homopurinic section, a central "hinge" made of four dT nucleotides, and a 12-nucleotide homopyrimidinic section ending at the 3′ terminus, capable of forming an intramolecular Watson–Crick duplex with 5′ homopurinic sequences. Oligo B was a 3′-thiolated oligonucleotide whose sequence was composed of a 12-nucleotide homopyrimidinic part and a 20-nucleotide-long poly-dA ending at the 3′ terminus. The

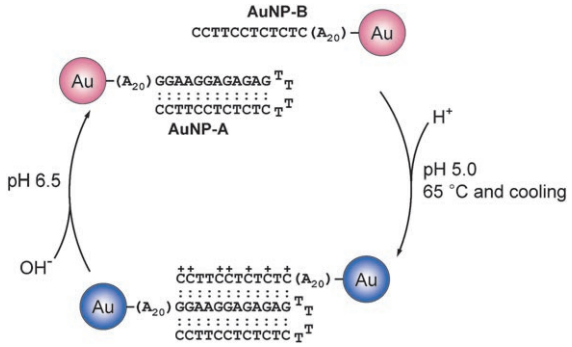

Figure 1. Triplex–AuNP conjugates and their assembly. The protonated cytosine is indicated by +C. One AuNP presented multiple copies of oligonucleotides at its surface; only one of these oligonucleotides is shown for simplicity. The triplex-driven assembly of AuNPs yielded three-dimensional networks of AuNPs.

thiol group was located at the 3′ terminus of oligo B, to form a parallel triplex between oligo A and oligo B. Before attachment to AuNPs, the oligo A solution was heated to 90 °C for 5 min, slowly cooled to room temperature, and left in an ice bath for oligo A to hybridize into the intramolecular duplex. The thiol-terminated oligonucleotides (oligo A and oligo B) were then treated with citrate-stabilized AuNPs (15 nm in diameter) in a buffered aqueous solution to obtain a dispersion of AuNPs bearing multiple oligonucleotide molecules per nanoparticle.

The proton-fueled assembly of the triplex–AuNP conjugates was investigated by first mixing together oligo A-attached (AuNP–A) and oligo B-attached AuNPs (AuNP–B) at pH 6.5 (50 mM sodium phosphate buffer containing 10 mM $MgCl_2$) and then acidifying the mixture until pH 5 was reached. The formation of a triplex requires a reduction in the repulsion between the negatively charged phosphate groups of the three strands. The repulsion between these phosphates can be effectively screened by divalent cations, such as Mg^{2+}.[10] The UV/Vis absorption spectrum was recorded before the acidification: the maximum absorbance wavelength (λ_{max}) was 525 nm, indicative of dissociated states of AuNPs (Figure 2). The mixed nanoparticles were assembled

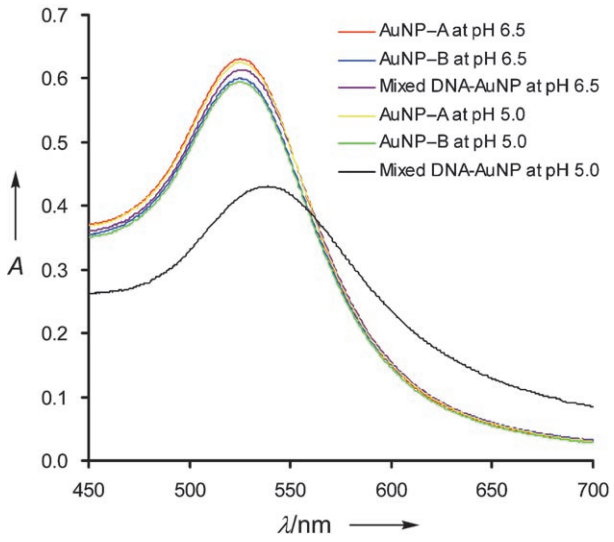

Figure 2. UV/Vis absorption spectra of AuNP–A, AuNP–B, and a mixture of AuNP–A and AuNP–B at pH 6.5 and 5.0.

by heating and slow cooling of the hybridized solution for 48 hours. During the assembly process, the solution changed from the initial red to a reddish-purple color, which indicated that a triplex was formed by two interacting oligonucleotides, oligo A and oligo B, attached to AuNPs. The UV/Vis absorption spectrum also confirmed the proton-fueled assembly of AuNPs; we observed spectral changes in the region of 450–700 nm as a result of the formation of nanoparticle aggregates following the hybridization of the triplex-forming oligonucleotides at pH 5 (Figure 2, black line).

The assembly process also led to a red shift in the surface plasmon resonance from 525 to 537 nm. This absorbance change was the result of the reduced inter-nanoparticle distances through triplex formation.[1,2] As a control experiment, each solution containing AuNP–A or AuNP–B was subjected to a change of pH value from 6.5 to 5, which did not lead to any changes in the UV/Vis spectra. The control experiment confirmed that the observed spectral change resulted from the proton-fueled formation of a triplex between AuNP–A and AuNP–B at pH 5.

Transmission electron microscopy (TEM) experiments were performed to characterize the morphologies of mixed DNA–AuNP composites at pH 6.5 (Figure 3a) and 5.0

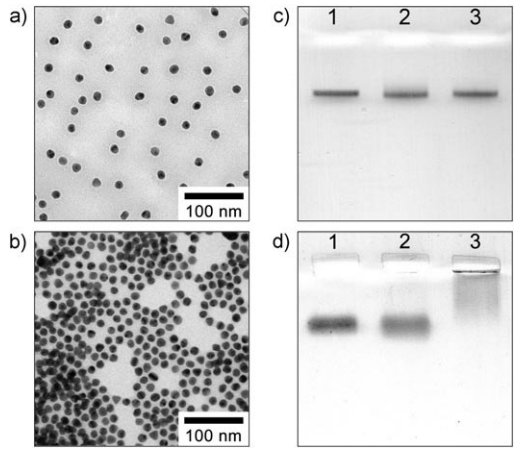

Figure 3. TEM images of mixtures of AuNP–A and AuNP–B at a) pH 6.5 and b) pH 5.0. c) Electrophoretic mobility of AuNP–A (lane 1), AuNP–B (lane 2), and mixed DNA–AuNP (lane 3) at pH 6.5. AuNP solutions were loaded onto agarose gels (1% w/v in 10 mM sodium phosphate, pH 6.5) and subjected to electrophoresis at 100 V for 30 min. d) Electrophoretic mobility of DNA–AuNP conjugates at pH 5.2. Sodium acetate (30 mM) was used instead of sodium phosphate.

(Figure 3b). The TEM image in Figure 3b confirmed the proton-fueled assembly of AuNPs, which indicated that the nanoparticles were linked to each other by hybridization of the triplex-forming oligonucleotide and the hairpin-structured Watson–Crick duplex.

To compare the electrophoretic mobility of triplex-driven aggregates with those of the triplex–AuNP conjugates (AuNP–A and AuNP–B) under the conditions for triplex formation, the resulting aggregate solution was analyzed by electrophoretic mobility shift assay using 1% agarose gel in sodium acetate (30 mM, pH 5.2) as an electrophoresis buffer (Figure 3d). The assembled structure (lane 3) moved slower than AuNP–A (lane 1) and AuNP–B (lane 2), and some aggregates were also observed to stay in the well. In contrast, all of the DNA–AuNP conjugates (AuNP–A, AuNP–B, and mixed DNA–AuNP) had the same mobility in gel electrophoresis at pH 6.5 (Figure 3c).

The repeated switching and pH-dependent reversible assembly/disassembly of triplex–AuNP conjugates were monitored with UV/Vis absorption spectroscopy. The absorbance of the mixed nanoparticle solution was measured while the pH of the sample was repeatedly cycled between 5.0 and

6.5 with controlled additions of 1M HCl and 1M NaOH. The change in λ_{max} value of the nanoparticle solutions was plotted with respect to pH value during six cycles starting at pH 6.5 (Figure 4). At pH 6.5, λ_{max} of the mixed nanoparticle solution

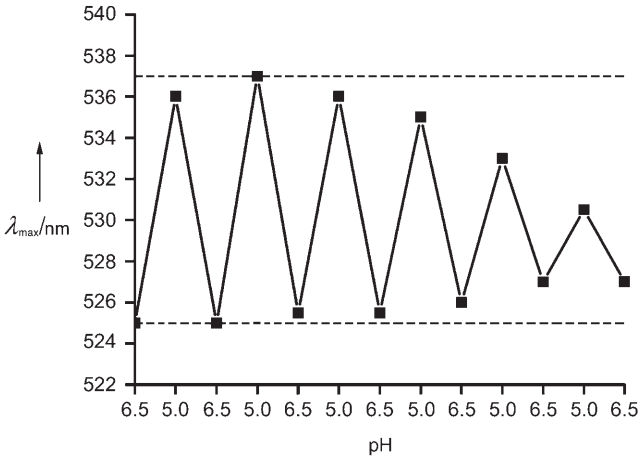

Figure 4. pH-dependent, reversible cycling of the assembly of triplex–AuNP conjugates observed by UV/Vis absorption spectroscopy. The change in the maximum absorbance wavelength (λ_{max}) of the nanoparticle solutions was plotted with respect to pH value. The initial concentration of each triplex–AuNP conjugate was sodium phosphate buffer (50 mM) with $MgCl_2$ (10 mM). The pH value was cycled between 5.0 and 6.5 by alternating addition of 1 M HCl or 1 M NaOH.

was about 525 nm. This λ_{max} value indicated the breakdown of the assembled network of AuNPs by triplex denaturation. Under hybridizing buffer conditions at pH 5.0, λ_{max} changed to about 537 nm. This shift in λ_{max} synchronized with the induced changes in pH value, and was continually observed during cycling. The gradual decrease in λ_{max} at pH 5.0 may have been caused by dilution of the nanoparticle solutions upon addition of acid or base, accumulated NaCl, and partial formation of irreversible aggregates. A gradual increase in the ionic strength as a result of the accumulated NaCl was expected to change the electrostatic potential of the triplex structure and consequently its performance.[10]

In summary, the protonation of the imino group (N3) of cytosines in double-stranded DNA that is required for the formation of a triplex was used to guide the assembly/disassembly of DNA-conjugated AuNPs. This proton-fueled switching of AuNP networks could be useful in various areas of nanobiotechnology. For example, it is possible that structures composed of triplex-forming AuNPs would generate swelling/shrinking of the whole structure in response to changes in pH value, and such variations might be applied to the design of a pH-dependent delivery system for small molecules, such as drugs and probe entities. In addition, a combination of the triplex-forming AuNP networks and other self-assembled nanostructures may lead to hybrids with a structure controlled by the pH-dependent structural change of triplexes.

Experimental Section

Oligonucleotides were synthesized by Genotech (Korea) on a 1-μmol scale. The thiol-modified oligonucleotides were 48- and 32-base oligonucleotides with the following sequences, respectively; oligo A: 5′-thiol(C_6)-A_{20}GG AAG GAG AGA GTT TTC TCT CTC CTT CC-3′ and oligo B: 5′-CCT TCC TCT CTC A_{20}-thiol(C_3)-3′. Gold colloids (15 nm in diameter) were purchased from BBInternational (UK).

Before using each oligonucleotide, a working stock solution was prepared with Tris–EDTA buffer (10 mM, pH 7.4). Dithiol linkages were cleaved prior to use by adding dithiothreitol (DTT; 1M, 40 μL) and sodium phosphate buffer (1M, 68 μL, pH 8.0) to a solution of the oligonucleotide (200 μL, 25 pmol μL^{-1}). Thiolated oligonucleotides were then desalted on a NAP-25 (Pharmacia, UK) column using sodium phosphate buffer (10 mM, pH 7.0) as the solvent. The resulting fractions were analyzed by UV/Vis absorption spectroscopy with a Shimadzu 2550 spectrophotometer. We added 4.8 nmol of the oligonucleotide to an AuNP solution (3 mL; 2.3 nM). The entire solution was covered with tin foil and stored at room temperature for 16 h. This solution was then brought to sodium phosphate buffer (10 mM, pH 7.0) containing sodium chloride (50 mM). The solution was shaken for another 48 h, and the salt concentration was increased gradually from 50 to 300 mM by adding 2 M NaCl. The solution was then centrifuged at 15000 g for 20 min. The supernatant containing starting material oligonucleotides was removed and the reddish solid at the bottom of the centrifuge tube was dispersed in sodium phosphate buffer (10 mM, pH 7.0). This procedure was repeated and the reddish solid obtained was finally redispersed in sodium phosphate buffer (10 mM, 1.5 mL, pH 7.0).

Hybridization and assembly experiments were carried out with 1 pmol of each triplex–AuNP conjugate (AuNP–A and AuNP–B) in sodium phosphate buffer containing $MgCl_2$. The conjugate was quickly mixed with the same volume of $MgCl_2$ (20 mM) and sodium phosphate (100 mM, pH 5.0). The mixed solution was heated to 65 °C for 5 min, slowly cooled to 37 °C, and then stored at 37 °C for 24 h and at 4 °C for 24 h.

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